22 January 2009

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Chem-Bio News– S&T Edition

1. SULFUR MUSTARD PENETRATION OF THERMOPLASTIC ELASTOMERS: *"To pass the Liquid Agent Vapour Penetration Test (LAVPT), Santoprene sheet needed to be 1.03 mm thick while Alcryn sheet needed to be 0.73 mm thick."*

2. DEVELOPMENT OF AN AUTOFLUORESCENT WHOLE-CELL BIOCATALYST BY DISPLAYING DUAL FUNCTIONAL MOIETIES ON ESCHERICHIA COLI CELL SURFACES AND CONSTRUCTION OF A COCULTURE WITH ORGANOPHOSPHATE-MINERALIZING ACTIVITY: *""Therefore, the coculture with autofluorescent and mineralizing activities can potentially be applied for bioremediation of OP-contaminated sites."*

3. DETECTION OF HUMAN ORTHOPOXVIRUS INFECTIONS AND DIFFERENTIATION OF SMALLPOX VIRUS WITH REAL-TIME PCR: *"We also demonstrated the applicability of the assay in human cases of cowpox and vaccinia virus infections."*

4. HEAT SHOCK INHIBITS CASPASE-1 ACTIVITY WHILE ALSO PREVENTING ITS INFLAMMASOME-MEDIATED ACTIVATION BY ANTHRAX LETHAL TOXIN: *"Our results suggest that heat shock inhibition of active caspase-1 can occur independently of an inflammasome platform, through a titratable factor present within intact, functioning heat-shocked cells."*

5. THE BACILLUS ANTHRACIS SLEL (YAAH) PROTEIN IS AN N-ACETYLGLUCOSAMINIDASE INVOLVED IN SPORE CORTEX DEPOLYMERIZATION:
"In the absence of the sleL-encoded N-acetylglucosaminidase, other cortex-lytic enzymes break down the cortex peptidoglycan sufficiently to allow rapid germination and outgrowth."

CB Daily Report

Chem-Bio News

SULFUR MUSTARD PENETRATION OF THERMOPLASTIC ELASTOMERS

By P. Miller
Defence Science and Technology Office (AS)
October, 2008

“Abstract

Resistance to HD penetration was investigated for two commercially available thermoplastic elastomers, Santoprene and Alcryn. To pass the Liquid Agent Vapour Penetration Test (LAVPT), Santoprene sheet needed to be 1.03 mm thick while Alcryn sheet needed to be 0.73 mm thick. Therefore, to pass the LAVPT, Alcryn sheet could be 29% thinner than Santoprene sheet.

Executive Summary

The resistance to sulfur mustard (HD) penetration of two thermoplastic elastomers, Santoprene and Alcryn, was investigated. Most military style CBR (chemical biological radiological) respirators are fabricated from thermosetting elastomers such as butyl rubber or silicone rubber. Compared to thermoplastic elastomers, these thermosetting elastomers are expensive and difficult to process. Therefore a thermoplastic elastomer that could provide sufficient levels of penetration resistance would have great potential as a replacement for butyl and silcone in CBR respirators. To pass the Liquid Agent Vapour Penetration Test (LAVPT), Santoprene sheet needed to be at least 1.03 mm thick while Alcryn sheet needed to be at least 0.73 mm thick. Therefore, to pass the LAVPT, Alcryn sheet could be 29% thinner than Santoprene sheet. A number of compounds, including fluorinated additives, activated carbon and talc powder, were added to the thermoplastic elastomers to improve their resistance to chemical penetration. Further development of this research is recommended to study: the effect of the inclusion of additives on the mechanical properties of the material; investigation of the thermal properties of component materials and investigation into processing polymer/activated carbon black composites.”

The full article can be found at: http://dspace.dsto.defence.gov.au/dspace/bitstream/1947/9668/1/DSTO-TN-0846%20PR.pdf

Return to Top

DEVELOPMENT OF AN AUTOFLUORESCENT WHOLE-CELL BIOCATALYST BY DISPLAYING DUAL FUNCTIONAL MOIETIES ON ESCHERICHIA COLI CELL SURFACES AND CONSTRUCTION OF A COCULTURE WITH ORGANOPHOSPHATE-MINERALIZING ACTIVITY

Proteomics Weekly
January 19, 2009

"Surface display of the active proteins on living cells has enormous potential in the degradation of numerous toxic compounds. Here, we report the codisplay of

organophosphorus hydrolase (OPH) and enhanced green fluorescent protein (GFP) on the cell surface of Escherichia coli by use of the truncated ice nucleation protein (INPNC) and Lpp-OmpA fusion systems."

"The surface localization of both INPNC-OPH and Lpp-OmpA-GFP was demonstrated by Western blot analysis, immunofluorescence microscopy, and a protease accessibility experiment. Anchorage of GFP and OPH on the outer membrane neither inhibits cell growth nor affects cell viability, as shown by growth kinetics of cells and stability of resting cultures. The engineered E. coli can be applied in the form of a whole-cell biocatalyst and can be tracked by fluorescence during bioremediation. This strategy of codisplay should open a new dimension for the display of multiple functional moieties on the surface of a bacterial cell. Furthermore, a coculture comprised of the engineered E. coli and a natural p-nitrophenol (PNP) degrader, Ochrobactrum sp. strain LL-1, was assembled for complete mineralization of organophosphates (OPs) with a PNP substitution. The coculture degraded OPs as well as PNP rapidly."

"Therefore, the coculture with autofluorescent and mineralizing activities can potentially be applied for bioremediation of OP-contaminated sites."

The full article can be found at: (C. Yang, et. al., "Development of an Autofluorescent Whole-Cell Biocatalyst by Displaying Dual Functional Moieties on Escherichia coli Cell Surfaces and Construction of a Coculture with Organophosphate-Mineralizing Activity". Applied and Environmental Microbiology, 2008;74(24):7733-7739). Link not available.

Return to Top

DETECTION OF HUMAN ORTHOPOXVIRUS INFECTIONS AND DIFFERENTIATION OF SMALLPOX VIRUS WITH REAL-TIME PCR
Health Risk Factor Week
January 20, 2009

"We developed a real-time PCR protocol to detect orthopoxviruses (OPVs) from different clinical specimens and to separate variola virus from other OPVs. In our protocol, we used automated nucleic acid extraction system together with real-time PCR to create a simple, safe and fast procedure to obtain an initial result."

"The sensitivity was better by using designed hybridization probes as compared to SYBR green I for detection. The detection limit ranged from 13 to 1,300 copies per 20 mu l reaction volume depending on the sample type. The PCR detected all OPVs pathogenic to human (variola, cowpox, monkey-pox, vaccinia) as well as camel pox and ectromelia viruses. Amplification of variola virus sequences could be distinguished from other OPVs by melting curve analysis."

"We also demonstrated the applicability of the assay in human cases of cowpox and vaccinia virus infections."

The full article can be found at: (N. Putkuri, et. al., "Detection of Human Orthopoxvirus

Infections and Differentiation of Smallpox Virus With Real-Time PCR". Journal of Medical Virology, 2009;81(1):146-152). Link not available.

Return to Top

HEAT SHOCK INHIBITS CASPASE-1 ACTIVITY WHILE ALSO PREVENTING ITS INFLAMMASOME-MEDIATED ACTIVATION BY ANTHRAX LETHAL TOXIN
Preventive Medicine Week
January 18, 2009

""Anthrax lethal toxin (LT) rapidly kills macrophages from certain mouse strains in a mechanism dependent on the breakdown of unknown protein(s) by the proteasome, formation of the Nalp1b (NLRP1b) inflammasome and subsequent activation of caspase-1. We report that heat-shocking LT-sensitive macrophages rapidly protects them against cytolysis by inhibiting caspase-1 activation without upstream effects on LT endocytosis or cleavage of the toxin's known cytosolic substrates (mitogen-activated protein kinases)."

"Heat shock protection against LT occurred through a mechanism independent of de novo protein synthesis, HSP90 activity, p38 activation or proteasome inhibition and was downstream of mitogen-activated protein kinase cleavage and degradation of an unknown substrate by the proteasome. The heat shock inhibition of LT-mediated caspase-1 activation was not specific to the Nalp1b (NLRP1b) inflammasome, as heat shock also inhibited Nalp3 (NLRP3) inflammasome-mediated caspase-1 activation in macrophages. We found that heat shock induced pro-caspase-1 association with a large cellular complex that could prevent its activation. Additionally, while heat-shocking recombinant caspase-1 did not affect its activity in vitro, lysates from heat-shocked cells completely inhibited recombinant active caspase-1 activity."

"Our results suggest that heat shock inhibition of active caspase-1 can occur independently of an inflammasome platform, through a titratable factor present within intact, functioning heat-shocked cells."

The full article can be found at: (T.C. Levin, et. al., "Heat shock inhibits caspase-1 activity while also preventing its inflammasome-mediated activation by anthrax lethal toxin". Cellular Microbiology, 2008;10(12):2434-2446). Link not available.

Return to Top

THE BACILLUS ANTHRACIS SLEL (YAAH) PROTEIN IS AN N-ACETYLGLUCOSAMINIDASE INVOLVED IN SPORE CORTEX DEPOLYMERIZATION
Medical Letter on the CDC & FDA
January 18, 2009

"Bacillus anthracis spores, the infectious agents of anthrax, are notoriously difficult to remove from contaminated areas because they are resistant to many eradication methods.

These resistance properties are due to the spore's dehydration and dormancy and to the multiple protective layers surrounding the spore core, one of which is the cortex."

"In order for B. anthracis spores to germinate and resume growth, the cortex peptidoglycan must be depolymerized. This study reports on analyses of sleL (yaaH), which encodes a cortex-lytic enzyme. The inactivation of sleL does not affect vegetative growth, spore viability, or the initial stages of germination, including dipicolinic acid release. However, mutant spores exhibit a slight delay in the loss of optical density compared to that of wild-type spores. Mutants also retain more diaminopimelic acid and N-acetylmuramic acid during germination than wild-type spores, suggesting that the cortex peptidoglycan is not being hydrolyzed as rapidly. This finding is supported by high-pressure liquid chromatography analysis of the peptidoglycan structure used to confirm that SleL acts as an N-acetylglucosaminidase. When sleL is inactivated, the cortex peptidoglycan is not depolymerized into small muropeptides but instead is retained within the spore as large fragments."

"In the absence of the sleL-encoded N-acetylglucosaminidase, other cortex-lytic enzymes break down the cortex peptidoglycan sufficiently to allow rapid germination and outgrowth."

The full article can be found at: (E.A. Lambert, et. al., “The Bacillus anthracis SleL (YaaH) Protein Is an N-Acetylglucosaminidase Involved in Spore Cortex Depolymerization”. Journal of Bacteriology, 2008;190(23):7601-7607). Link not available.

Return to Top

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